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N-acetylcysteine as a Provisional, Commercial Off-The-Shelf (COTS) Chemoprotectant Against Sulfur Mustard

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PREFACE

This review was conducted at the Naval Health Research Center Detachment Toxicology (NHRC/TD) under the direction of CDR Warren W. Jederberg, MS, MSC, USN, Officer-in-Charge NHRC/TD. It was performed as a part of research funded by the Office of Naval Research under Work Unit # N61153.M04508.518.60263.

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N-acetylcysteine as a Provisional, Commercial Off-The-Shelf (COTS) Chemoprotectant Against Sulfur Mustard.

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Abstract

Sulfur mustard is a vesicant with a long history of use in conflict. It produces coalescing blisters of the skin, inflicts temporary or permanent blindness through profound irritation or corrosion of the eyes and produces dramatic lung injury. Extensive exposure can destroy the immune system by destruction of bone marrow cells. There is no antidote for HD, or effective treatment other than rapid decontamination and supportive care. Current development plans schedule a licensed HD prophylaxis in the FY10-19 range.

N-acetylcysteine (NAC) is a derivative of the amino acid cysteine which is rapidly converted to cysteine in the body. While it is classified as a "dietary supplement," it has been used clinically for over thirty years, primarily intravenously and orally in the treatment of acetaminophen overdose. Due to its support of the glutathione system, one of the primary physiological detoxification pathways, it has been tested recently for a number of clinical activities. Placebo-controlled, double-blind studies have shown it to provide clinical benefit for: prevention of influenza symptoms; slowing of Alzheimer's progression; liver function restoration following septic shock; acute respiratory distress; and ocular symptoms of Sjögren's Syndrome. Animal and tissue studies have suggested efficacy for NAC as a chemoprotectant against acrylamide, asbestos, cigarette smoke, catecholamines, diesel exhaust, free radicals, and methoxyacetic acid. Other animal studies indicate a possible role for NAC in the treatment of porphyria, brain injury, and anthrax.

Recent animal studies have demonstrated the ability of NAC to significantly decrease lung damage from HD and a HD simulant. Tissue culture studies indicated that NAC may also decrease the damage of HD to skin and lymphocytes.

NAC is a compound of extremely low toxicity. Common intravenous doses are 150 mg/kg. The minimum toxic dose reported in a human is 8480 mg/kg by mixed routes.

The US Armed Forces are currently preparing for possible war against an enemy with a documented history of using HD in battle. Given the historical effectiveness of HD on the battlefield, the lack of any effective treatment, the demonstrated general chemoprotective properties of NAC, the overall safety of NAC administration, the lack of a licensure requirement, and the preliminary data supporting efficacy against HD exposure, we suggest the daily oral administration of the maximum known safe dose of NAC to all US forces entering combat zones.

Additional studies should be performed to better demonstrate efficacy against moderate HD exposure in animal models, and to identify longer-term activities.

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The Threat

History of Use

Sulfur Mustard (bis-(2-chloroethyl) sulfide; CAS 505-60-2) is often referred to by the acronym HD; while HD technically refers to a highly purified form of sulfur mustard generally produced after WWI, in this communication it will be used, for brevity's sake, to refer to any sulfur mustard formulation. HD was first used in war near Ypres, Belgium on July 12, 1917; Although it was only used for one year, HD was responsible for about 80% of the chemical casualties in WWI. Italy used mustard on the Abyssinians (Ethiopians) in its 1935-36 conquest of that country, and Japan used it in China starting in 1939 (Smart 1997). Mustard was inadvertently released from the *SS John* Harvey in 1943 by German bombing (Alexander, 1947).HD, probably of Soviet make, was used by Egypt in the 1963-67 Yemen War (Shoham 1998). Iraq used mustard (probably HD) extensively in the Iran-Iraq war (CIA 2002), causing as many as 45,000 casualties (Carus 1988).

Mustard Effects

HD is a vesicant, that is, a blister agent. It is an oily liquid of low volatility which causes extensive damage to all contacted tissues. The skin develops erythema typically 4 to 8 hours after exposure. Vesication appears 2 to 18 hours later (Sidell *et al*, 1997); this delay of effects promotes the spreading of the agent beyond the original deployment site. These blisters will generally coalesce over time to produce large bulla 0.5 to 5 cm in diameter. Liquid mustard may produce a full-thickness (third degree) burn. Severe skin lesions may require months to heal (Willems 1989). Inhaled mustard vapor can produce acute airway injury 4 to 6 hours after exposure, including hoarseness, pharyngeal and laryngeal erythema, wheezing and dyspnea, and epithelial sloghing with pseudomembrane formation in severe exposures (Sidell *et al*, 1997).

Initial nonbacterial bronchitis is followed by bacterial superinfection in 4 to 6 days. Ocular exposure to even small amounts of mustard vapor result in irritation; more severe exposures bring on pain, severe conjunctivitis, and corneal damage (Sidell *et al*, 1997). HD-induced photophobia lasts for weeks. Most deaths result from pulmonary damage complicated by infection, and occur several days following exposure.

Two hypotheses exist for the biochemical effects of mustard exposure. HD is a DNA alkylating agent (Papirmeister *et al*, 1985), causing strand breaks which are irreparable to cellular machinery (Sidell *et al*, 1997). The normal response to irreparable DNA damage is apoptosis, sometimes called programmed cell death, in which the cell actively destroys itself through a series of metabolic processes including DNA segmentation, proteolysis of cellular enzymes, and disruption of the cell membrane (Li, 1999). This is consistent with reports that many HD effects appear to be mediated through apoptotic mechanisms (Dabrowska *et al*, 1996; Atkins *et al*, 2000; Kehe *et al*, 2000; Blaha *et* al 2001; Rosenthal *et al*, 2001, 2003). Secondly, since HD reacts rapidly with glutathione, an intracellular reducing agent involved in many detoxification reactions (Gentilhomme *et al*, 1992), a second hypothesis for HD effects is the profound depletion of intracellular glutathione, leaving the cell susceptible to naturally-produced reactive oxygen species (Sidell *et al*, 1997). Damage from reactive oxygen species also results in apoptosis (Mates and Sanchez-Jimenez, 1999). It therefore appears that whatever the mechanism, 1) apoptosis is induced as the mechanism of tissue destruction, and 2) glutathione is a sink for HD damage, reducing the effective dose received by the cell and reducing the damage caused.

MEDICAL TREATMENT

There is currently no antidote or chemoprophylaxis for HD. The only means of reducing injury is decontamination of the exposure site within two minutes (Sidell, 1997); after this time, the

mustard has been absorbed and conjugated to biomolecules, and no benefit will be realized from decontamination. Following development of symptoms, only supportive care is available, such as ventilation, antibiotics, and skin burn care. The Department of Defense Chemical and Biological Defense Program identifies the goal of having a licensed chemoprophylaxis, but not until the FY10-19 period (DASDCBD, 2002).

N-acetylcysteine

NAC Structure and Metabolism

N-acetyl cysteine (CAS 616-91-1), or NAC, is a synthetic cysteine derivative (Figure 1). Although it has been used in clinical settings for many years (see below), its mechanism of action for observed clinical benefit have been identified much more recently.

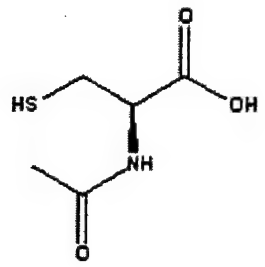

Figure 1: N-Acetylcysteine structure.

Glutathione (GSH) is a tripeptide made up of residues of glutamine, glycine, and cysteine (Figure 2). It is the principal thiol participant in cellular redox reactions, and part of the major cellular detoxification pathway; it is involved in the detoxification of most endogenous and exogenous toxicants (Sies 1999). Glutathione is also important in the pathology of cancer, AIDS, and other diseases (Voehringer 1999). Its principal activities are the reduction of reactive oxygen species (ROS) and free radicals (Ketterer, 1998), and s-conjugation leading to the increased export of conjugates both from the cell and from the body (Keppler, 1999). GSH is synthesized from its component amino acids via γ-glutamyl-cysteine synthase and glutathione synthase (Griffith 1999). The limiting factor for this synthesis is the concentration of cysteine, which can be 10 to 20-fold less than the concentration of the other amino acids (Griffith 1999). Therefore in

many cases the limiting factor in cellular detoxification capacity is the intracellular concentration of cysteine.

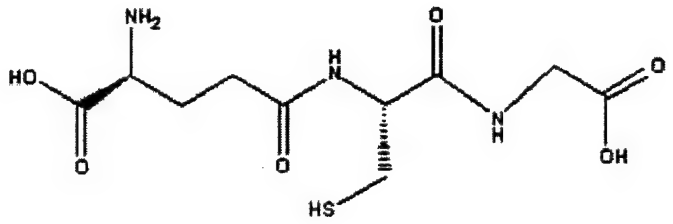

Figure 2: Glutathione Structure.

NAC can be given orally or intravenously and is readily deacetylated *in vivo* to physiological cysteine, whereas administered cysteine tends to auto-oxidize to an insoluble product (Griffith 1999). NAC is absorbed rapidly, if not efficiently, in humans; peak plasma concentration is achieved within 1 to 2 hours after a 200 to 400 mg oral dose. Most of the absorbed NAC is rapidly bound to protein or excreted; reduced NAC has a physiological half-life of 6.25 hours (Holdiness 1991). NAC does make an efficient physiological substrate for glutathione synthesis: preincubation of cultured hamster cells with NAC prevents depletion of GSH by toxicants (Park *et al*, 2002); in rats, NAC has been demonstrated to increase intracellular glutathione in erythrocytes, liver, and lung cells, reduce the activation of procarcinogens by the liver, and detoxicfication of mutagens (DeFlora *et al*, 1985); in humans, oral NAC administration increases GSH levels in T cells of AIDS patients (DeRosa *et al*, 2000). NAC is also able to substitute for GSH as a substrate for microsomal Glutatione-S-Transferase, a major detoxifying enzyme (Weinander *et al*, 1994). Given the importance of glutathione in cellular response to xenobiotics, free radicals, reactive oxygen species, and other sources of cellular damage, it should be obvious *a priori* that NAC should relieve or prevent a variety of human pathologies. And there

is a large volume of solid experimental evidence that NAC is clinically efficacious as chemical antidote and immune support.

DEMONSTRATED CLINICAL BENEFITS

NAC has been used for over 25 years as an antidote to acetaminophen overdose, both in oral and intravenous form (Smilkstein *et al*, 1988). It functions by replenishing, and substituting for, GSH in detoxifying the hepatotoxic metabolite of acetaminophen, N-Acetyl-p-benzoquinonimine. NAC has also been used in nebulized form, as a mucolytic agent, cleaving the sulfhydryl bonds in pulmonary mucus (Kasielski and Nowak, 2001)) Within the last decade, however, a number of new clinical uses for systemic NAC treatment have been tested in randomized, placebo-controlled, double-blind (RPD) studies.

Since the discovery that HIV-infected patients are deficient in glutathione (Buhl *et al*, 1989), several groups have evaluated the efficacy of NAC in the treatment of HIV and AIDS patients. Åkerlund *et al* (1996) in an RPD study found somewhat equivocal results with a daily dose of 800 mg for 4 months, although the dose was well tolerated. Treatment normalized plasma cysteine levels, slowed the decline of CD4+ lymphocytes, and decreased plasma TNF-α levels. Beitkreutz *et al* (2000) found better results with RPD symptomatic patients: a significant improvement of immunological function, if no change in viral load. DeRosa *et al* (2000) administered large doses (median dose 5.3 g/day for a median 24 weeks) and restored GSH levels in CD4 and CD8 T cells; they also saw a dramatic improvement in 2-year survival, but this was in an open-label phase of the test, potentially confounded by self-selection.

RPD systemic NAC trials for treatment of acute lung injury showed an improved systemic oxygenation and reduced the need for ventilatory support after 3 days of 40mg/kg/d iv NAC (Suter *et al*, 1994). A similar study with more seriously ill patients (Domenighetti *et al*,

1997) using 190 mg/kg/d NAC found no change in ventilator need or mortality, but some improvement in lung injury. A 6 month 1200 mg/d RPD study of 262 subjects, mostly over 65, found that the rate of seroconversion towards A/H_1N_1 Singapore 6/86 influenza was similar among study patients regardless of treatment; however, 75% of NAC-treated seoconverters did so asymptomatically, while only 21% of placebo-receiving seroconverers did not show symptoms (DeFlora *et al*, 1997). The NAC treatment also improved function of the patients' immune cells in antigen challenge tests. Twenty-six patients with Sjögren's Syndrome involved in a RPD cross-over study of NAC demonstrated that NAC relieves many of the subjective ocular symptoms, if not the objective laboratory tests (Walters *et al*, 1986). NAC increases liver blood flow following septic shock (Rank *et al*, 2000), and one recent RPD study (Adair *et al*, 2001) showed efficacy for NAC in the treatment of cognitive impairment in Alzheimer's disease.

CHEMOPROTECTION IN CELL CULTURE AND ANIMAL STUDIES

An even broader array of studies have been performed in cell cultures. Catecholamines are natural neurotransmitters used by the central and peripheral nervous systems; however, auto-oxidation produces free radicals which can be cytotoxic to the nerve cells. Out of a laundry list of antioxidants, only NAC prevents this chatecholamine toxicity in cortical cell cultures (Noh *et al*, 1999). Nitric Oxide (NO) donors are used as experimental tools for evaluating the effect of exogenously produced NO; they also result in glutathione depletion and cell death by apoptosis (Babich and Zuckerbraun, 2001); cotreatment with NAC prevents both effects. Zn2+ causes profound morphological changes, production of reactive oxygen species (ROS) and cell death in isolated rat neural cells; NAC co-treatment inhibited all of these effects (Ryu *et al*, 2002).Diesel exhaust causes cultured human bronchial cells to produce IL-8, a cytokine which usually promotes apoptosis (Abe *et al*, 2000); NAC significantly inhibited IL-8 production. Acrylamide is

tumorogenic in rats and mice, and promotes morphological transformation of Syrian hamster embryo (SHE) cells (Park *et al*, 2001), an indicator of carcinogenic potential; NAC reduces cellular transformation of SHE below control levels, even in the presence of a moderate amount of acrylamide. Pretreatment with NAC prevents the destruction of isolated rat testicular germ cells by methoxyacetic acid (MAA, a paint industry byproduct), suggesting its ability to prevent human testicular atrophy, the main hazard of MAA (Rao and Shaha, 2002).

NAC has been demonstrated in animals to have chemoprotectant properties against a number of hazardous chemicals. At approximately 1g/kg/d, oral NAC prevented bronchial epithelial thickening/ hyperplasia (Rogers and Jeffery, 1986), and reduced DNA damage (Izzoti *et al*, 2001) in smoke-exposed rats. NAC moderated the effects of asbestos exposure in rat lungs (Afaq *et al*, 2000), accelerated healing of free radical-damaged soft tissue (van der Laan *et al*, 1997), and even protected against the lethal effects of perfluoroisobutene (PFIB) in rats (Lailey 1997; van de Meent *et al*, unpublished results).

OTHER NAC ACTIVITIES

Owing to its support of cellular physiology and suppression of apoptosis, NAC also appears to have activity against other types of pathology. NAC enhances the inhibition of prostaglandin synthesis by NSAIDs in isolated monocytes (Hoeffer *et al*, 2002). 5-Aminolevulinic acid (ALA) is a product of metabolism which is part of the pathology of porphyria and lead poisoning, accumulating intracellularly and producing free radicals which damage DNA; NAC treatment in cell culture prevented DNA damage (Yusof *et al*, 1999). 163 mg/kg NAC given intraperitoneally before or immediately after fluid-percussion brain injury to cats maintained the normal arteriolar constriction response to hyperventilation, whereas control

animals lost this response after injury (Ellis *et al*, 1991). NAC was even able to partially protect mice against challenge with anthrax lethal toxin (Hanna *et al*, 1994).

MUSTARD CHEMOPROTECTION

Although it had been known for some time that HD formed glutathione conjugates (Kinsey and Grant, 1947), research in NAC chemoprotection against HD agent began about 15 years ago when it was noted that resistance to nitrogen mustard (used as cancer chemotherapy) could be correlated to glutathione levels, and that depletion of glutathione levels would render tumors sensitive to mustard treatment (Ono and Shrieve, 1987).Gross *et al* later demonstrated (1993) that pretreatment of human peripheral blood lymphocytes with 10 mM NAC reduced the effects of lower concentrations of HD, but not high concentrations, and the suggestion was made at that time that augmentation of intracellular glutathione may provide some protection against HD agent. A later study using vascular endothelial cells in a model of capillary leakage and edema (Dabrowska *et al*, 1996) found that NAC pretreatment nearly eliminated apoptotic effects, although it did little against necrotic effects; since most clinical effects appear to be related to apoptosis, this finding is supportive of chemoprotection. The same group later demonstrated that the effect is primarily due to enhanced glutathione synthesis (Atkins *et al,* 2000). Similar experiments using bronchial epithelial cells found NAC co-treatment (NAC administration simultaneous with HD exposure) provided significant, although by no means complete, protection against HD, but pretreatment was not tested (Rappeneau *et al*, 2000).

Anderson *et al* (2000) tested co-treatment in rats for protection from HD vapors. NAC was able to virtually eliminate many of the effects of HD exposure within the first 24 hours. Considering that intraperitoneal injection requires time for absorption in to the mesenteric capillary system and that HD is typically combined or metabolized within the first few minutes

after exposure, this is a significant finding. McClintock *et al* (2002), in the most recent study, found that NAC reduced lung injury by 70% if administered 10 minutes prior to HD exposure, and had significant benefit if administered up to 90 minutes after exposure.

Combined, the animal and cell data is very promising that NAC should have some benefit to individuals exposed to HD in the field. To be properly cautious, it should be noted that the animal studies described here focus on low to moderate lung exposure, so few conclusions can be drawn about NAC's ability to ameliorate more serious exposures. However, even in an attack the majority of HD exposures are likely to be lower level, and the protection of these personnel would be a significant benefit to US forces. It is also impossible to predict from these studies the impact of NAC on skin exposure, except to note that NAC does affect glutathione levels in the skin.

NAC SAFETY

N-acetylcysteine has one of the lowest toxicities of known chemicals. Its probable lethal dose is 5-15 g/kg body weight (Gosselin *et al*, 1984), or about 3.3 pounds for the average person. It is commonly packaged as a 600 mg capsule for unregulated sales. As acetaminophen antidote, it is commonly delivered intravenously as a 20% solution, leading to several grams run in over a period of 15 minutes. Cases of toxicity from this regimen are rare (ex: Reynard *et al*, 1992), and complicated by symptoms from the overdose (Ellenhorn and Barceloux, 1988). No systematic experiments were identified in a Medline/ Toxline literature search to determine maximum tolerated dose in humans or in animals.

To answer the question of a responsible long-term maximum tolerated dose there are two studies to consider: a study of 262 mostly older (78% $\geq$ 65 yrs) who took 1200 mg/day for six months (166 $\pm$ 35 days) reported a level of adverse events (9%, mostly mild gastrointestinal

effects) not significantly higher than the placebo group (DeFlora *et al*, 1997); 64 HIV infected patients took a median dose of 5300 mg/d for a similar length of time (median 24 weeks) (DeRosa *et al*, 2000) and saw no difference between NAC and placebo in the rate of study withdraw for perceived side effects. To base the dose on systemic effects, the latter, higher-dose study would be appropriate; however, delivery was by effervescent tablet, which contained a large amount of bicarbonate. On the other hand, the study participants of both studies were likely to be less resistant to gastrointestinal insults and any other negative effects than healthy military personnel. A dose of 4800 mg/day (8 standard 600 mg capsules distributed over the day; 90% of the HIV study median dose) has a very low risk of significant adverse effects, with the most likely being nausea or diarrhea. In the event such effects occur, they should be rectifiable by reducing the dose.

Data Limitations and Research Needs

In order to obtain the maximum benefit from NAC, DOD should invest in a number of studies to further evaluate its potential and dosing for maximum protection. While the list is long, a number of these studies can be, and in some cases, should be, performed concurrently and with little additional cost.

Oral dose studies. While intraperitoneal route is a simulation of the oral route, the dynamics of absorption can be significantly different, and such dosing may be more or less effective.

Animal pretreatment studies. Glutathione levels appear to build over time in response to oral NAC, but current studies initiate NAC treatment simultaneously with exposure, reflecting a "treatment" rather than prophylactic approach. There are no studies where animals are given oral NAC significantly prior to exposure.

Skin Efficacy Studies. As indicated above, there is little evidence to determine if NAC pretreatment would be effective against skin effects of mustard. Isolated skin models and, ultimately, whole-animal exposures need to be performed to determine efficacy.

Dose-effect studies. Studies need to be performed to establish a relationship between NAC dose and response to HD to determine the limits of NAC protection.

Long-term influence of NAC. Current NAC studies follow animals for less than 24 hours. Some studies suggest that preventing apoptosis does not prevent cell death over the longer term in all cases (Brown and Wouters, 1999). Exposed and protected animals need to be followed for longer periods of time to demonstrate an effect of NAC on clinical outcome, including recovery from acute injury.

Animal Tolerance Study. A more effective study of the maximum tolerated oral dose in animals needs to be conducted for extrapolation to humans, to determine if the dose can be safely raised for healthy individuals.

NAC Toxicity Study. A long-term animal study of high and subtoxic dosed animals to determine the targets of acute and chronic NAC toxicity, to include neurological testing. This would also involve a NAC toxicity curve, to determine the difference between a minimally toxic and potentially lethal dose.

Human tolerance study. Ultimately the dose should be recalculated based on the animal tolerance studies using the toxicity curve to determine the appropriate safety margin and a test using volunteers in a double-blind, placebo-controlled performed, looking exclusively at safety of dosing.

Summary and Conclusions

The United States is currently preparing for armed conflict in the Persian Gulf. Given the potential for, and history of, the use of HD by enemy forces, consideration must be given to the protection of our forces from unexpected HD attack. While there is too little time for research to provide specific solutions; any potential Commercial Off-The-Shelf (COTS) solution should be seriously considered.

HD attacks mammalian cells by causing DNA lesions which cannot be easily corrected, leading to cell death and, if widespread enough, loss of organ (lung, skin, immune system) function. The principal cellular defense against these effects is direct detoxification of HD by glutathione conjugation prior to DNA damage. Consequently, the increase of glutathione activity, either through glutathione augmentation or by providing a physiologically effective substitute, is reasonably the best means of providing cellular-level protection to fielded forces from the effects of sudden HD exposure.

N-acetylcysteine has been used for decades to boost liver glutathione activity in the event of acetaminophen overdose, and is effective in both the intravenous and oral forms. It is clear from the variety of other clinical and experimental effects NAC demonstrates that its activity is quite wide-reaching, including, at a minimum, the lungs, brain, testes, and immune system. Its safety in moderate dose is unquestionable, owing to the long history of use, and it has been used in weakened populations at high dose for relatively long periods of time with no noted ill effects.

NAC is defined as a dietary supplement despite its clinical history, and as such is not an investigational new drug and does not fall under the licensure requirements of a prescription medication. It should therefore be available to dispense to combat forces immediately, with no legal or ethical limitations other than prudent dose assignment.

N-acetylcysteine is a potential COTS chemoprotectant against HD, a threat for which there is currently no antidote, and no plans for a licensed chemoprotectant until after FY10.

Literature Cited

Abe S, Takizawa H, Sugawara I, Kudoh S. 2000. Diesel exhaust (DE)-induced cytokine expression in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 22: 296-303

Adair JC, Knoefel JE, Morgan N. 2001. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's Disease. *Neurology* 57: 1515-7

Afaq F, Abidi P, Rahman Q. 2000. N-acetyl L-cysteine attenuates oxidant-mediated toxicity induced by chrysotile fibers. *Toxicol Lett* 117: 53-60

Åkerlund B, Jarstrand C, Lindeke B, Sönnerborg A, Åkerblad A-C, Rasool O. 1996. Effect of N-acetylcysteine (NAC) treatment on HIV-1 infection: a double-blind placebo-controlled trial. *Eur J Clin Pharmacol* 50:457-61

Alexander SF. 1947. Medical Report of the Bari Harbor Mustard Casualties. *Milit. Surgeon* 101: 2- 17

Andreson DR, Byers SL, Vesely KR. Treatment of sulfur mustard (HD)-induced lung injury. *J Appl Toxicol* 20: S129-32

Atkins KB, Lodhi IJ, Hurley LL, Hinshaw DB. 2000. N-acetylcysteine and endothelial cell injury by sulfur mustard. *J Appl Toxicol* 20: S125-S128

Babich H, Zuckerbraun HL. 2001. In vitro cytotoxicity of glycol-S-nitrosothiols: a novel class of nitric oxide donors. *Toxicol in Vitro* 15: 181-190

Blaha M, Kohl J, DuBose D, Bowers W Jr, Walker J. 2001. Ultrastructural and histological effects of exposure to CEES or heat in a human epidermal model. *In Vitr Mol Toxicol* 14:15-23

Brown JM, Wouters BG. 1999. Apoptosis, p53, and tumor cell sensitivity to anticancer agents. *Cancer Res* 59: 1391-9

Buhl R, Holroyd KJ, Mastrangeli A, Cantin AM, Jaffe HA, Wells FB, Saltini C, Crystal RG. 1989. Systemic glutathione deficiency in symptom free HIV seropositive individuals. Lancet 334: 1294-8

Carus WS. 1988. *Chemical Weapons in the Middle East*. The Washington Institute for Near East Policy, Washington, DC

CIA. 2002. *Iraq's Weapons of Mass Destruction Programs*. Central Intelligence Agency unclassified report. Available at: http://www.cia.gov/cia/publications/iraq_wmd/Iraq_Oct_2002.pdf

Dabrwoska MI, Becks LL, Lelli JL Jr, Levee MG, Hinshaw DB. 1996. Sulfur mustard induces apoptosis and necrosis in endothelial cells. *Toxicol Appl Pharmacol* 141: 568-583

DASDCBD (Deputy Assistant to the Secretary of Defense for Chemical and Biological Defense). 2002. *Department of Defense Chemical and Biological Defense Program. Volume I: Annual report to Congress*. Office of the Secretary of Defense.
Available online at http://www.acq.osd.mil/cp/nbc02/vol1-2002cbdpannualreport.pdf

DeFlora S, Bennicelli C, Camoiriano A, Serra D, Romano M, Rossi GA, Morelli A, DeFlora A. 1985. In vivo effects of N-acetylcysteine on glutathione metabolism and on the biotransformation of carcinogenic and/or mutagenic compounds. *Carcinogenesis* 6: 1735-45

DeFlora S, Grassi C, Carati L. 1997. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. *Eur Respir J* 10:1535-1541

DeRosa SC, Zaretsky MD, Dubs JG, Roederer M, Anderson M, Green A, Mitra D, Watanabe N, Nakamura H, Tijoe I, Deresinski SC, Moore WA, Ela SW, Parks D, and Herzenberg LA. N-acettlcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest* 30: 915-929

Domenighetti G, Suter PM, Schaller MD, Ritz R, Perret C. 1997. Treatment with N-acetylcysteine during acute respiratory distress syndrome: a randomized, double-blind, placebo-controlled clinical study. *J Crit Care* 12: 177-82

Ellenhorn MJ, Barceloux DG. 1988. *Medical Toxicology-Diagnosis and Treatment of Human Poisoning*. Elsevier Science Publishing Co, New York. P163

Ellis EF, Dodson LY, Police RJ. 1991. Restoration of cerebrovascular responsiveness to hyperventilation by the oxygen radical scavenger n-acetylcysteine following experimental traumatic brain surgery. *J Neurosurg* 75: 774-9

Elsayed NM, Omaye ST, Klain GJ, Inase JL, Dahlberg ET, Wheeler CR, Korte DW Jr. 1989. Response of mouse brain to a single subcutaneous injection of the monofunctional sulfur mustard, butyl 2-chloroethyl sulfide (BCS). *Toxicol* 58: 11-20

Gentilhomme E, Neveux Y, Hua A, Thiriot C, Faure M, Thivolet J. 1992. Action of bis(betachloroethyl) sulphide (BCES) on human epidermis reconstituted in culture: Morphological alterations and biochemical depletion of glutathione. *Toxicol in Vitro* 6: 139-47

Gosselin RE, Smith RP, Hodge HC. *Clinical Toxicology of Commercial Products, 5th ed.* Williams and Wilkins, Baltimore, 1984.

Griffith OW. 1999. Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Rad Biol Med* 27: 922-35

Gross CL, Innace JK, Hovatter RC, Meier HL, Smith WJ. 1993. Biochemical manipulation of intracellular glutathione levels influences cytotoxicity to isolated lymphocytes by sulfur mustard. *Cell Biol Toxicol* 9: 259-267

Hanna PC, Kruskal BA, Ezekowitz RA, Bloom BR, Collier RJ. 1994. Role of macrophage oxidative burst in the action of anthrax lethal toxin. *Mol Med* 1:7-18

Hoffer E, Baum Y, Nahir AM. 2002. N-Acetylcysteine enhances the action of anti-inflammatory drugs as suppressors of prostaglandin production in monocytes. *Mediators Inflamm* 11:321-3

Izzotti A, Balansky RM, D'Agnostini F, Bennicelli C, Myers SR, Grubbs CJ, Lubet RA, Kelloff GJ, DeFlora S. 2001. Modulation of biomarkers by chemopreventive agents in smoke-exposed rats. *Cancer Res* 61: 2472-9

Kasielski M, Nowak D. 2001. Long-term administration of N-acetylcysteine decreases hydrogen peroxide exhalation in subjects with chronic obstructive pulmonary disease. *Respir Med* 95: 448-56

Kehe K, Reisinger H, Szinicz L. 2000. Sulfur mustard induces apoptosis and necrosis in SCL II cells in vitro. *J Appl Toxicol* 20(S1):S81-S86

Keppler D. 1999. Export pumps for glutathione S-conjugates. *Free Radic Biol Med* 27:985-991

Ketterer B. 1998. Glutathione S-transferases and prevention of cellular free radical damage. *Free Radic Res* 28: 647-58

Kinsey VE, Grant WM. 1947. Action of mustard gas and other poisons on yeast cells III. Distribution of fixed mustard gas in yeast. *J Cell Comp Physiol* 29: 75-84

Lailey AF. 1997. Oral N-acetylcysteine protects against perfluoroisobutene toxicity in rats. *Hum Exp Toxicol* 16:212-

Li GM. 1999. The role of mismatch repair in DNA damage-induced apoptosis. *Oncol Res* 11:393-400

Mates JM, Sanchez-Jimenez FM.1999. Role of reactive oxygen species in apoptosis: implications for cancer therapy. *Int J Biochem Cell Biol* 32:157-70

McClintock SD, Till GO, Smith MG, Ward PA. 2002. Protection from half-mustard-gas-induced acute lung injury in the rat. *J Appl Toxicol* 22: 257-262

Noh JS, Kim EY, Kang JS, Kim HR, Oh YJ, Gwag BJ. 1999. Neurotoxic and neuroprotective actions of catecholamines in cortical neurons. *Exp Neurol* 159: 217-24

Ono K, Shrieve DC. 1987. Effect of glutathione depletion by L-buthione sulfoximine on the caytotoxicity of cyclophosphamide in single and fractionated doses to EMT6/SF mouse tumors and bone marrow. *J Natl Cancer Inst* 79: 811-5

Papirmeister B, Gross CL, Meier HL, Petrali JP, Johnson JB. 1985. Molecular basis for mustard-induced vessication. *Fund Appl Toxicol* 5: S134-49

Park J, Kamendulis LM, Friedman MA, Klaunig JE. 2002. Acrylamide-induced cellular transformation. *Toxicol Sci* 65: 177-83

Rank N, Michel C, Haertel C, Lenhart A, Welte M, Meier-Hellmann A, Spies C. 2000. N-acetylcysteine increases liver blood flow and improves liver function in septic shock patients: results of a prospective, randomized, double-blind study. *Crit Care Med* 28: 3799-807

Rao AVSK, Shaha C. 2002. N-acetylcysteine prevents MAA induced male germ cell apoptosis: role of glutathione and Cytochrome c. *FEBS Lett* 527:133-137

Rappeneau S, Baeza-Squiban A, Marano F, Clavet J-H. 2000. Efficient protection of human bronchial epithelial cells against sulfur and nitrogen mustard cytotoxicity using drug combinations. *Toxicol Sci* 58: 153-160

Rogers DF, Jeffrey PK. 1986. Inhibition by oral N-acetylcysteine of cigarette smoke-induced "bronchitis" in the rat. *Exp Lung Res* 10:267-83

Rosenthal DS, Velena A, Chou FP, Schlegel R, Ray R, Benton B, Anderson D, Smith WJ, Simbulan-Rosenthal CM. 2003. Expression of dominant-negative FADD blocks human keratinocyte apoptosis and vesication induced by sulfur mustard. *J Biol Chem.*, In press

Rosenthal DS, Simbulan-Rosenthal CM, Liu WF, Velena A, Anderson D, Benton B, Wang ZQ, Smith W, Ray R, Smulson ME. 2001. PARP determines the mode of cell death in skin fibroblasts, but not keratinocytes, exposed to sulfur mustard. *J Invest Dermatol.* 117:1566-73

Ryu R, Shin Y, Choi JW, Min W, Ryu H, Choi CR, Ko H. 2002. Depletion of intracellular glutathione mediates zinc-induced cell death in rat primary astrocytes. *Exp Brain Res* 143:257-63

Shoham D. 1998. Chemical and biological weapons in Egypt. *Nonprolif Rev* Spring-Summer 1998, 48-58

Sidell FR, Urbanetti JS, Smith WJ, Hurst CG. 1997. Vesicants. In *Medical Aspects of Chemical and Biological Warfare.* TMM Publications, Borden Institute, Walter Reed Army Medical Center, Washington, DC.

Sies H. 1999. Glutathione and its role in cellular functions. *Free Rad Biol Med* 27: 916-21

Smart JK. 1997. History of Chemical and Biological Warfare: an American Perspective. In *Medical Aspects of Chemical and Biological Warfare.* TMM Publications, Borden Institute, Walter Reed Army Medical Center, Washington, DC.

Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. 1988. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. *New Eng J Med* 319: 1557-62

Suter PM, Domenighetti G, Schaller MD, Laverriere MC, Ritz R, Perret C. 1994. N-acetylcysteine enhances recovery from acute lung injury in man. A randomized, double-blind, placebo-controlled clinical study. *Chest* 105:190-4

Van de Meent D, Oostdilk JP, Joosen MJA, Diemel RV, Kuijpers WC, van Helden HPM. Lung Injury caused by lung oedemagens: treatment with surfactant and anti-inflammatory drugs. Unpublished technical report. http://www.pmfhk.cz/RTO/TG-004/Papers/Helden.pdf.

Van der Laan L, Oyen WJG, Verhofstad AAJ, Tan ECTH, ter Laak HJ, Gabreels-Festen A, Hendriks T, Goris RJA. 1997. Soft tissue repair capacity after oxygen-derived free radical-induced damage in one hindlimb of the rat. *J of Surg Res* 72:60-69

Vijayaraghavan R, Sugendran K, Pant SC, Husain K, Malhotra RC. 1991. Dermal intoxication of mice with bis(2-chloroethyl)sulphide and the protective effect of flavinoids. *Toxicol* 69: 35-42

Voehringer DW. 1999. Bcl-2 and glutathione: alterations in cellular redox state that regulate apoptosis sensitivity. *Free Rad Biol Med* 27: 945-950

Walters MT, Rubin CE, Keightly SJ, Ward CD, Cawley MID. 1986. A double-blind, cross-over, study of oral N-acetylcysteine in Sjögren's Syndrome. *Scand J Rheumatol* 61: 252-8

Weinander R, Anderson C, Morgenstern R. 1994. Identification of N-acetylcysteine as a new substrate for rat liver microsomal glutathione transferase. A study of thiol ligands. *J Biol Chem* 269:71-6

Wilde PE, Upshall DG. 1994. Cysteine esters protect cutured rodent slices from sulphur mustard. *Hum Exp Toxicol* 13:743-8

Willems JL. 1989. Clinical Management of mustard gas casualties. *Ann Med Milit Belg* 3S:1-61

Yusof M, Yildiz D, Ercal N. 1999. N-acetyl-L-cysteine protects against δ-aminolevulinic acid-induced 8-hydroxydeoxyguanosine formation. *Toxicol Lett* 106:41-47

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13. **ABSTRACT** *(Maximum 200 words)* Sulfur mustard is a vesicant with a long history of use in conflict. It produces coalescing blisters of the skin, inflicts temporary or permanent blindness through profound irritation or corrosion of the eyes and produces dramatic lung injury. Extensive exposure can destroy the immune system by destruction of bone marrow cells. There is no antidote for HD, or effective treatment other than rapid decontamination and supportive care. Current development plans schedule a licensed HD prophylaxis in the FY10-19 range. Animal and tissue studies have suggested efficacy for NAC as a chemoprotectant against acrylamide, asbestos, cigarette smoke, catecholamines, diesel exhaust, free radicals, and methoxyacetic acid. Recent animal studies have demonstrated the ability of NAC to significantly decrease lung damage from HD and a HD simulant. Tissue culture studies indicated that NAC may also decrease the damage of HD to skin and lymphocytes. Given the historical effectiveness of HD on the battlefield, the lack of any effective treatment, the demonstrated general chemoprotective properties of NAC, the safety of NAC administration, the lack of a licensure requirement, and the data supporting efficacy against HD exposure, we suggest the daily oral administration of the maximum safe dose of NAC to personnel entering combat zones.

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